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STATISTICAL COMBINING OF CELL EXPRESSION PROFILES

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Exhibit A

10058696.012802

EXHIBIT A

MARKED UP VERSION OF REPLACEMENT PARAGRAPHS OF THE SPECIFICATION

U.S. PATENT APPLICATION SERIAL NO. TO BE ASSIGNED, DIVISIONAL OF U.S. PATENT APPLICATION SERIAL NO. 09/222,596

On page 1, line 1, please insert the following paragraph:

<u>**CROSS REFERENCE TO RELATED APPLICATION**</u>

<u>This application is a division of United States Application no. 09/222,596, filed December 28, 1998, which is incorporated by reference herein in its entirety.</u>

On page 7, please replace the paragraph beginning "Fig. 1 depicts" with the following paragraph:

Fig. 1 depicts some sources of measurement error present in microarray fluorescent images. [(A)] <u>Panel (a)</u> depicts unevenly printed DNA probe spots. [(B)] <u>Panel (b)</u> depicts the effects of scratches, dust, and artifacts. [(C)] <u>Panel (c)</u> depicts how spot positions drift away from a nominal measuring grid. [(D)] <u>Panel (d)</u> depicts the effects of unevenness in the brightness across the microarray due to uneven hybridization. [(E)] <u>Panel (e)</u> depicts the effects of color stripes on the microarray due to fluorophore-specific biases.

On page 8, please replace the paragraph beginning "Fig. 4A is a color ratio" with the following paragraph:

Fig. [4A] <u>4a</u> is a color ratio vs. intensity plot for an experiment in which both cultures were the same background strain of the yeast S. <u>cerevisiae</u> [Cerevisiae]. Genes with a distinct bias between a red and green fluorophore are flagged. Fig. [4B] <u>4b</u> is the same experiment as depicted in Fig. [4A] <u>4a</u> except that usage of the red and green fluorophores is reversed. [Fig 4C] <u>Fig. 4c</u> depicts the bias removal process of the invention, wherein [Fig4A] <u>Fig. 4a</u> and

[Fig4B] Fig. 4b are combined to produce a response profile free of fluorophore-specific biases.

On page 11, please replace the paragraph beginning "Microarrays are advantageous" with the following paragraph:

Microarrays are advantageous because nucleic acids representing two different pools of nucleic acid can be hybridized to a microarray and the relative signal from each pool can simultaneously be measured. Each [of] pool of nucleic acids may represent the state of a biological system before and after a perturbation. For example, a first nucleic acid pool may be derived from a mRNA pool from a cell culture before exposing the cell culture to a pharmacological agent and a second cDNA pool may be derived from a mRNA pool derived from the same culture after exposing the culture to a pharmacological agent. Alternatively, the two pools of cDNA could represent pathway responses. Thus, a first cDNA library could be derived from the mRNA of a first aliquot ("pool") of a cell culture that has been exposed to a pathway perturbation and a second cDNA library can be derived from the mRNA of a second aliquot ("pool") of the same cell culture wherein the second aliquot was not exposed to the pathway perturbation. As used herein, microarray experiments, including those described in this section, are referred to as ("differential microarray experiments"). One skilled in the art will appreciate that many forms of differential microarray experiments other than the ones outlined in this disclosure are within the scope of the definition of "differential microarray experiments". Further, as used herein, the term "differential intensity measurement" refers to measurements made in differential microarray experiments. For example, a differential intensity measurement could be the difference between the brightness of a position on a microarray, which corresponds to a cellular constituent of interest, after (i) the microarray has been contacted with DNA derived from a biological system that represents a baseline state and (ii) the microarray has been contacted with DNA derived from a biological system that represents a perturbed state. Further, one skilled in the art will appreciate that the baseline state of a biological system may represent the wild-type state of the biological system. Alternatively, the baseline state of a biological system could represent a different perturbed state of the biological system. Each microarray experiment in a

differential microarray experiment, or repeated differential microarray experiment preferably utilizes the same or similar microarray. Microarrays are considered similar if they are prepared from substantially isogenic biological systems and a majority of the binding spots on each microarray are common. Thus, the microarray used in repeated microarray experiments may be the same identical microarray, wherein the microarray is washed between microarray experiments, or the microarray(s) used in repeated microarray experiments may be exact replicas of each other, or they may be similar to each other.

On page 13, please replace the paragraph beginning "Cell Expression Profiles" with the following paragraph, (in which insertions are shown by double underlining):

Cell Expression Profiles An advantage of using two different cDNA pools in microarray experiments is that a direct and internally controlled comparison of the mRNA levels corresponding to each arrayed gene in two cell states can be made. This and related techniques for quantitative measurement of cellular constituents [constitutents] is generally referred to as cell constituent profiling. Cell constituent profiling is typically expressed as changes, either in absolute level or the ratio of levels, between two known cell conditions, such as a response to treatment of a baseline state with a pharmacological agent, as described in the previous section.

On page 17, please replace the paragraph beginning "If a gene of interest is present in the top 5%" with the following paragraph:

If a gene of interest is present in the top 5% of up regulations in a first and second nominal repeat of a microarray experiment, the chance that it appeared that up regulated by chance in both arrays is only 0.05 * 0.05 = .0025 or .25%, assuming systematic biases have been removed. Thus repeating the measurement allows a much higher level of confidence in declaring that the gene of interest is up regulated. In general, if expression ratios in any number of repeated experiments are expressed as percentile rankings, the chance $P(H_0)$ that any (pre-specified) gene of interest is not actually up regulated is

$$P(H_0^+) = \prod_i P_i \qquad (5)$$

where P_i is the percentile rank in the $\underline{i^{th}}$ [i'th] experiment, expressed as a fraction (fifth percentile = 0.05). The probability that the gene is not *down*-regulated is given by

$$P(H_0^-) = \prod_i (1 - P_i) \qquad (6)$$

These rank-based methods provide a powerful way of reducing false alarms with repeated measurements. For example, setting a threshold at the upper 5% of expression ratios in a hybridization to probes covering the yeast genome, which has approximately 6000 genes, would yield ~6000*-0.05 = 300 false detections in a single experiment, but less than one false detection on average if the same 5% threshold were applied across four experiment repeats $(6000*(0.05)^4)$. This rank combining has the advantage that it does not require any modeling of the detailed error behavior in the underlying hybridization experiments, other than the assumption of no systematic biases. The rank based method is an example of a non-parametric statistical test for the significance of observed up- or down- regulations.

On page 23, please replace the paragraph beginning "The use of genesets for representing projected profiles" with the following paragraph:

The use of genesets for representing projected profiles is described in this and the following subsections and also detailed in U.S. Patent application serial number 09/179,569 filed October 27, 1998, now U.S. Patent No. 6,203,987 dated March 20, 2001, entitled "Methods for using co-regulated genesets to enhance determination and classification of gene expression" by Friend *et al.*, and U.S. patent application serial number 09/220,275 [to be assigned] (Attorney docket number 9301-039-999) filed December 23, 1998 by Friend *et al.*, entitled "Methods for using co-regulated genesets to enhance determination and classification of gene expression" which are both incorporated herein by reference in their entireties. Certain genes tend to increase or decrease their expression in groups. Genes tend to increase or decrease their rates of transcription together when they possess similar regulatory sequence patterns, *i.e.*, transcription factor binding sites. This is the mechanism for coordinated response to particular signaling inputs (*see, e.g.*, Madhani and Fink, 1998, The riddle of MAP

kinase signaling specificity, Transactions in Genetics 14:151-155; Arnone and Davidson, 1997, The hardwiring of development: organization and function of genomic regulatory systems, Development 124:1851-1864). Separate genes which make different components of a necessary protein or cellular structure will tend to co-vary. Duplicated genes (*see, e.g.*, Wagner, 1996, Genetic redundancy caused by gene duplications and its evolution in networks of transcriptional regulators, Biol. Cybern. 74:557-567) will also tend to co-vary to the extent mutations have not led to functional divergence in the regulatory regions. Further, because regulatory sequences are modular (*see, e.g.*, Yuh *et al.*,1998, Genomic cis-regulatory logic: experimental and computational analysis of a sea urchin gene, Science 279:1896-1902), the more modules two genes have in common, the greater the variety of conditions under which they are expected to co-vary their transcriptional rates. Separation between modules also is an important determinant since co-activators also are involved. In summary therefore, for any finite set of conditions, it is expected that genes will not all vary independently, and that there are simplifying subsets of genes and proteins that will co-vary. These co-varying sets of genes form a complete basis in the mathematical sense with which to describe all the profile changes within that finite set of conditions.

On page 38, please replace the paragraph beginning "Microarrays were images on a prototype" with the following paragraph:

Microarrays were images on a prototype multi-frame CCD camera in development at Applied Precision, Inc. (Seattle, WA). Each CCD image frame was approximately 2mm square. Exposure time of 2 sec in the Cy5 channel (white light through Chroma 618-648 nm excitation filter, Chroma 657-727 nm emission filter) and 1 sec in the Cy3 channel (Chroma 535-560 nm excitation filter, Chroma 570-620 nm emission filter) were done consecutively in each frame [fram] before moving to the next, spatially contiguous frame. Color isolation between the Cy3 and Cy5 channels was ~100:1 or better. Frames were knitted together in software to make the complete images. The intensity of spots (~100μm) were quantified from the 10 μm pixels by frame background subtraction and intensity averaging in each channel. Dynamic range of the resulting spot intensities was typically a ratio [ration] of 1000 between the brightest spots and the background-subtracted [background-subracted] additive

error level. Normalization between the channels was accomplished by normalizing each channel to the mean intensities of all genes. This procedure is nearly equivalent to normalization between channels using the intensity ratio [ration] of genomic DNA spots (See DeRisi *et al.*, 1997), [(See DeRisi *et al.*, 1997) ,] but is possibly more robust since it is based on the intensities of several thousand spots distributed over the array.

Exhibit B

10058696.012802

EXHIBIT B

CLEAN COPY OF REPLACEMENT PARAGRAPHS OF THE SPECIFICATION U.S. PATENT APPLICATION SERIAL NO. TO BE ASSIGNED, DIVISIONAL OF U.S. PATENT APPLICATION SERIAL NO. 09/222,596

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CROSS REFERENCE TO RELATED APPLICATION

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$$P(H_0^+) = \prod_i P_i \qquad (5)$$

where P_i is the percentile rank in the i^{th} experiment, expressed as a fraction (fifth percentile = 0.05). The probability that the gene is not *down*-regulated is given by

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These rank-based methods provide a powerful way of reducing false alarms with repeated measurements. For example, setting a threshold at the upper 5% of expression ratios in a hybridization to probes covering the yeast genome, which has approximately 6000 genes, would yield ~6000*-0.05 = 300 false detections in a single experiment, but less than one false detection on average if the same 5% threshold were applied across four experiment repeats ($6000*(0.05)^4$). This rank combining has the advantage that it does not require any modeling of the detailed error behavior in the underlying hybridization experiments, other than the assumption of no systematic biases. The rank based method is an example of a non-parametric statistical test for the significance of observed up- or down- regulations.

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genomic DNA spots (See DeRisi *et al.*, 1997), but is possibly more robust since it is based on the intensities of several thousand spots distributed over the array.

Exhibit C

EXHIBIT C

MARKED VERSION OF THE CLAIMS

AMENDMENT (Filed *DRAFT*)

U.S. PATENT APPLICATION SERIAL NO. TO BE ASSIGNED, DIVISIONAL OF U.S. PATENT APPLICATION SERIAL NO. 09/222,596

14. (Amended) A method for determining a probability that an expression level of a cellular constituent in a plurality of paired differential microarray experiments is altered by a perturbation, wherein each paired differential microarray experiment in said plurality of paired differential microarray experiments comprises a first microarray experiment representing a baseline state of a first biological system, and a second microarray experiment representing a perturbed state of said first biological system, said method comprising the steps of

(a) determining an error distribution statistic by fitting a reference pair of microarray experiments with an intensity independent statistic, wherein said reference pair of microarray experiments comprises a first reference microarray experiment, and a second reference microarray experiment that is a nominal repeat of said first reference microarray experiment;

(b) [selecting said cellular constituent from a set of cellular constituents measured in said plurality of paired differential microarray experiments, and, for each paired differential microarray experiment in said plurality of paired differential microarray experiments,] determining, for each paired differential microarray experiment in said plurality of paired differential microarray experiments, an amount of change in expression level of said cellular constituent between the second microarray experiment and the first microarray experiment of said paired differential microarray experiment using said error distribution statistic; and

(c) determining said probability that said expression level of said cellular constituent in said plurality of paired differential microarray experiments is altered by said perturbation by combining, for each paired differential microarray experiment in said plurality of paired differential microarray experiments, [said] each amount of change in expression level of said cellular constituent determined in step (b) [for each paired differential microarray experiment in said plurality of paired differential microarray experiments] using a rank based method.

15. (Amended) A computer system for determining a probability that an expression level of a cellular constituent in a plurality of paired differential microarray experiments is altered by a perturbation, wherein each paired differential microarray experiment in said plurality of paired differential microarray experiments comprises a first microarray experiment representing a baseline state of a first biological system, and a second microarray experiment representing a perturbed state of said first biological system; the computer system comprising a processor, and a memory encoding one or more programs coupled to the processor and the one or more programs cause the processor to perform a method comprising the steps of

(a) determining an error distribution statistic by fitting a reference pair of microarray experiments with an intensity independent statistic, wherein said reference pair of microarray experiments comprises a first reference microarray experiment, and a second reference microarray experiment that is a nominal repeat of said first reference microarray experiment;

(b) [selecting said cellular constituent from a set of cellular constituents measured in said plurality of paired differential microarray experiments, and, for each paired differential microarray experiment in said plurality of paired differential microarray experiments,] determining<u>, for each paired differential microarray experiment in said plurality of paired differential microarray experiments,</u> an amount of change in expression level of said cellular constituent between the second microarray experiment and the first microarray experiment [of said paired differential microarray experiment] using said error distribution statistic; and

(c) determining said probability that said expression level of said cellular constituent in said plurality of paired differential microarray experiments is altered by said perturbation by combining<u>, for each paired differential microarray experiment in said plurality of paired differential microarray experiments,</u> [said] <u>each</u> amount of change in expression level of said cellular constituent determined in step (b) [for each paired differential microarray experiment in said plurality of paired differential microarray experiments] using a rank based method.

16. (Amended) The method of Claim 14 wherein said error distribution statistic is calculated according to a formula

$$\frac{(X - Y)}{\sqrt{\sigma_X^2 + \sigma_Y^2 + f^2(X^2 + Y^2)}}$$

where X represents an intensity of said [a] cellular constituent in said first microarray experiment of said reference pair of microarray experiments, Y represents an intensity of said cellular constituent in said second microarray experiment of said reference pair of microarray experiments, σ_X^2 is a variance term for X that represents an additive error level in X, σ_Y^2 is a variance term for Y that represents an additive error level in Y, and f is a fractional multiplicative error level.

17. (Amended) The computer system of Claim 15 wherein said error distribution statistic is calculated according to a formula

$$\frac{(X - Y)}{\sqrt{\sigma_X^{\ 2} + \sigma_Y^{\ 2} + f^2(X^2 + Y^2)}}$$

where X represents an intensity of said [a] cellular constituent in said first microarray experiment of said reference pair of microarray experiments, Y represents an intensity of said cellular constituent in said second microarray experiment of said reference pair of microarray experiments, σ_X^2 is a variance term for X that represents an additive error level in X, σ_Y^2 is a variance term for Y that represents an additive error level in Y, and f is a fractional multiplicative error level.

18. (Amended) The method of Claim 16 wherein said rank based method comprises determining a rank for said amount of change in expression level of said cellular constituent between said second microarray experiment and said first microarray experiment of said paired differential microarray experiment in relation to all cellular constituent [contituents] measurements in said plurality of paired differential microarray experiments using said error distribution statistic [according to a magnitude derived by the formula of Claim 16].

19. (Amended) The computer system of Claim 17 wherein said rank based method comprises determining a rank for said amount of change in expression level of said cellular constituent between said second microarray experiment and said first microarray experiment of said paired differential microarray experiment in relation to all cellular constituent [contituents]

measurements in said plurality of paired differential microarray experiments using said error distribution statistic [according to a magnitude derived by the formula of Claim 17].

20. (Amended) The method of Claim 14 wherein said rank based method determines a probability that said [a] cellular constituent is up-regulated in response to a perturbation.

21. (Amended) The computer system of Claim 15 wherein said rank based method determines a probability that said [a] cellular constituent is up-regulated in response to a perturbation.

22. (Amended) The method of Claim [20] 14 wherein said rank based method comprises computing [has the form]

$$P(H_0^+) = \prod_i P_i$$

where P_i is the percentile rank of the expression of said cellular constituent in the i^{th} pair [said probability that a cellular constituent is up-regulated in said plurality] of paired differential microarray experiments in said plurality of paired differential microarray experiments [experiment i, i is a paired differential microarray experiment selected from said plurality of paired differential microarray experiments, P is said probability that said expression level of said cellular constituent is up-regulated in response to said perturbation] , and $P(H_0^+)$ is the chance that said cellular constituent is not up-regulated in said plurality of paired differential microarray experiments.

23. (Amended) The method of Claim 14 wherein said rank based method determines a probability that said [a] cellular constituent is down-regulated in response to [a] said perturbation.

24. (Amended) The method of Claim [23] 14 wherein said rank based method comprises computing [has the form]

$$P(H_0^-) = \prod_i (1 - P_i)$$

where P_i is the percentile rank of the expression of said cellular constituent in the i^{th} pair [said probability that a cellular constituent is down-regulated in] of paired differential microarray experiments in said plurality of paired differential microarray experiments [experiment i, is selected from said plurality of paired differential microarray experiments, and P is said probability that said cellular constituent is down-regulated in response to said perturbation] , and $P(H_0^-)$ is the chance that said cellular constituent is not up-regulated in said plurality of paired differential microarray experiments.

25. (Amended) The method of Claim 14 wherein each paired differential microarray experiment in said plurality of paired differential microarray experiments is a two-fluorophore microarray experiment [experiments] wherein a first fluorophore represents said baseline state of said biological system and a second fluorophore, distinguishable from said first fluorophore, represents said perturbed state of said biological system.

26. (Amended) The method of Claim 14 wherein a single fluorophore is used in each said paired differential microarray experiments in said plurality of paired differential microarray experiments.

27. (Amended) The method of Claim 14 wherein a first fluorophore [label] is used in said first reference microarray experiment and a second fluorophore, distinguishable from said first fluorophore, [label] is used in said second reference microarray experiment.

Exhibit D

EXHIBIT D

THE CLAIMS WHICH WILL BE PENDING UPON ENTRY OF THE PRESENT AMENDMENT (Filed *DRAFT*)

U.S. PATENT APPLICATION SERIAL NO. TO BE ASSIGNED, DIVISIONAL OF U.S. PATENT APPLICATION SERIAL NO. 09/222,596

14. (Amended) A method for determining a probability that an expression level of a cellular constituent in a plurality of paired differential microarray experiments is altered by a perturbation, wherein each paired differential microarray experiment in said plurality of paired differential microarray experiments comprises a first microarray experiment representing a baseline state of a first biological system, and a second microarray experiment representing a perturbed state of said first biological system, said method comprising the steps of

(a) determining an error distribution statistic by fitting a reference pair of microarray experiments with an intensity independent statistic, wherein said reference pair of microarray experiments comprises a first reference microarray experiment, and a second reference microarray experiment that is a nominal repeat of said first reference microarray experiment;

(b) determining, for each paired differential microarray experiment in said plurality of paired differential microarray experiments, an amount of change in expression level of said cellular constituent between the second microarray experiment and the first microarray experiment of said paired differential microarray experiment using said error distribution statistic; and

(c) determining said probability that said expression level of said cellular constituent in said plurality of paired differential microarray experiments is altered by said perturbation by combining, for each paired differential microarray experiment in said plurality of paired differential microarray experiments, each amount of change in expression level of said cellular constituent determined in step (b) using a rank based method.

15. (Amended) A computer system for determining a probability that an expression level of a cellular constituent in a plurality of paired differential microarray experiments is altered by a perturbation, wherein each paired differential microarray experiment in said plurality of

paired differential microarray experiments comprises a first microarray experiment representing a baseline state of a first biological system, and a second microarray experiment representing a perturbed state of said first biological system; the computer system comprising a processor, and a memory encoding one or more programs coupled to the processor and the one or more programs cause the processor to perform a method comprising the steps of

(a) determining an error distribution statistic by fitting a reference pair of microarray experiments with an intensity independent statistic, wherein said reference pair of microarray experiments comprises a first reference microarray experiment, and a second reference microarray experiment that is a nominal repeat of said first reference microarray experiment;

(b) determining, for each paired differential microarray experiment in said plurality of paired differential microarray experiments, an amount of change in expression level of said cellular constituent between the second microarray experiment and the first microarray experiment using said error distribution statistic; and

(c) determining said probability that said expression level of said cellular constituent in said plurality of paired differential microarray experiments is altered by said perturbation by combining, for each paired differential microarray experiment in said plurality of paired differential microarray experiments, each amount of change in expression level of said cellular constituent determined in step (b) using a rank based method.

16. (Amended) The method of Claim 14 wherein said error distribution statistic is calculated according to a formula

$$\frac{(X - Y)}{\sqrt{\sigma_X{}^2 + \sigma_Y{}^2 + f^2(X^2 + Y^2)}}$$

where X represents an intensity of said cellular constituent in said first microarray experiment of said reference pair of microarray experiments, Y represents an intensity of said cellular constituent in said second microarray experiment of said reference pair of microarray experiments, $\sigma_X{}^2$ is a variance term for X that represents an additive error level in X, $\sigma_Y{}^2$ is a variance term for Y that represents an additive error level in Y, and f is a fractional multiplicative error level.

17. (Amended) The computer system of Claim 15 wherein said error distribution statistic is calculated according to a formula

$$\frac{(X - Y)}{\sqrt{\sigma_X{}^2 + \sigma_Y{}^2 + f^2(X^2 + Y^2)}}$$

where X represents an intensity of said cellular constituent in said first microarray experiment of said reference pair of microarray experiments, Y represents an intensity of said cellular constituent in said second microarray experiment of said reference pair of microarray experiments, $\sigma_X{}^2$ is a variance term for X that represents an additive error level in X, $\sigma_Y{}^2$ is a variance term for Y that represents an additive error level in Y, and f is a fractional multiplicative error level.

18. (Amended) The method of Claim 16 wherein said rank based method comprises determining a rank for said amount of change in expression level of said cellular constituent between said second microarray experiment and said first microarray experiment of said paired differential microarray experiment in relation to all cellular constituent measurements in said plurality of paired differential microarray experiments using said error distribution statistic.

19. (Amended) The computer system of Claim 17 wherein said rank based method comprises determining a rank for said amount of change in expression level of said cellular constituent between said second microarray experiment and said first microarray experiment of said paired differential microarray experiment in relation to all cellular constituent measurements in said plurality of paired differential microarray experiments using said error distribution statistic.

20. (Amended) The method of Claim 14 wherein said rank based method determines a probability that said cellular constituent is up-regulated in response to a perturbation.

21. (Amended) The computer system of Claim 15 wherein said rank based method determines a probability that said cellular constituent is up-regulated in response to a perturbation.

22. (Amended) The method of Claim 14 wherein said rank based method comprises computing

$$P(H_0^+) = \prod_i P_i$$

where P_i is the percentile rank of the expression of said cellular constituent in the i^{th} pair of paired differential microarray experiments in said plurality of paired differential microarray experiments, and $P(H_0^+)$ is the chance that said cellular constituent is not up-regulated in said plurality of paired differential microarray experiments.

23. (Amended) The method of Claim 14 wherein said rank based method determines a probability that said cellular constituent is down-regulated in response to said perturbation.

24. (Amended) The method of Claim 14 wherein said rank based method comprises computing

$$P(H_0^-) = \prod_i (1 - P_i)$$

where P_i is the percentile rank of the expression of said cellular constituent in the i^{th} pair of paired differential microarray experiments in said plurality of paired differential microarray experiments, and $P(H_0^-)$ is the chance that said cellular constituent is not up-regulated in said plurality of paired differential microarray experiments.

25. (Amended) The method of Claim 14 wherein each paired differential microarray experiment in said plurality of paired differential microarray experiments is a two-fluorophore microarray experiment wherein a first fluorophore represents said baseline state of said biological system and a second fluorophore, distinguishable from said first fluorophore, represents said perturbed state of said biological system.

26. (Amended) The method of Claim 14 wherein a single fluorophore is used in each said paired differential microarray experiments in said plurality of paired differential microarray experiments.

27. (Amended) The method of Claim 14 wherein a first fluorophore is used in said first reference microarray experiment and a second fluorophore, distinguishable from said first fluorophore, is used in said second reference microarray experiment.

43. (New) The method of Claim 14 wherein said perturbed state of said first biological system is achieved by a method comprising exposing said first biological system, when representing said baseline state, to a pharmacological agent.

44. (New) The computer system of Claim 15 wherein said perturbed state of said first biological system is achieved by a method comprising exposing said first biological system, when representing said baseline state, to a pharmacological agent.

45. (New) The method of Claim 14 wherein said perturbed state of said first biological system is achieved by a method comprising exposing said first biological system, when representing said baseline state, to a drug candidate.

46. (New) The computer system of Claim 15 wherein said perturbed state of said first biological system is achieved by a method comprising exposing said first biological system, when representing said baseline state, to a drug candidate.

47. (New) The method of Claim 14 wherein said perturbed state of said first biological system is achieved by a method comprising introducing an exogenous gene into said first biological system.

48. (New) The computer system of Claim 15 wherein said perturbed state of said first biological system is achieved by a method comprising introducing an exogenous gene into said first biological system.

49. (New) The method of Claim 14 wherein said perturbed state of said first biological system is achieved by a method comprising deleting a gene from said first biological system.

50. (New) The computer system of Claim 15 wherein said perturbed state of said first biological system is achieved by a method comprising deleting a gene from said first biological system.

51. (New) The method of Claim 14 wherein said perturbed state of said first biological system is achieved by a method comprising changing a culture condition of said first biological system.

52. (New) The computer system of Claim 15 wherein said perturbed state of said first biological system is achieved by a method comprising changing a culture condition of said first biological system.

53. (New) The method of Claim 14 wherein said perturbed state of said first biological system is due to the onset of a disease in said first biological system.

54. (New) The computer system of Claim 15 wherein said perturbed state of said first biological system is due to the onset of a disease in said first biological system.

55. (New) The method of Claim 14 wherein said first biological system is a cell line, a cell culture, a tissue sample, an organ, or a multicellular organism.

56. (New) The computer system of Claim 15 wherein said first biological system is a cell line, a cell culture, a tissue sample, an organ, or a multicellular organism.

57. (New) The method of Claim 14 wherein said first biological system is a mammal.

58. (New) The computer system of Claim 15 wherein said first biological system is a mammal.

59. (New) The method of Claim 14 wherein said first biological system is a *Homo sapien*.

60. (New) The computer system of Claim 15 wherein said first biological system is a *Homo sapien*.

61. (New) The method of Claim 14 wherein said first biological system is a yeast that is substantially isogenic to *Saccharomyces cerevisia.*

62. (New) The computer system of Claim 15 wherein said first biological system is a yeast that is substantially isogenic to *Saccharomyces cerevisia.*

63. (New) The method of Claim 14 wherein said baseline state represents the wild-type state of said first biological system.

64. (New) The computer system of Claim 15 wherein said baseline state represents the wild-type state of said first biological system.

65. (New) The method of Claim 14 wherein said baseline state represents a different perturbed state of said first biological system.

66. (New) The computer system of Claim 15 wherein said baseline state represents a different perturbed state of said first biological system.

67. (New) The method of Claim 14 wherein each said first microarray experiment and each said second microarray experiment in each paired differential microarray experiment in said plurality of paired differential microarray experiments uses a microarray having binding sites corresponding to at least fifty percent of the genes in the genome of said first biological system, and wherein said first biological system is a cell or a multicellular organism.

68. (New) The computer system of Claim 15 wherein each said first microarray experiment and each said second microarray experiment in each paired differential microarray experiment in said plurality of paired differential microarray experiments uses a microarray having binding sites corresponding to at least fifty percent of the genes in the genome of said first biological system, and wherein said first biological system is a cell or a multicellular organism.

69. (New) The method of Claim 14 wherein each said first microarray experiment and each said second microarray experiment in each paired differential microarray experiment in said plurality of paired differential microarray experiments uses a microarray having binding sites corresponding to at least seventy-five percent of the genes in the genome of said first biological system, and wherein said first biological system is a cell or a multicellular organism.

70. (New) The computer system of Claim 15 wherein each said first microarray experiment and each said second microarray experiment in each paired differential microarray experiment in said plurality of paired differential microarray experiments uses a microarray having binding sites corresponding to at least seventy-five percent of the genes in the genome of said first biological system, and wherein said first biological system is a cell or a multicellular organism.

71. (New) The method of Claim 14 wherein each said first microarray experiment and each said second microarray experiment in each paired differential microarray experiment in said plurality of paired differential microarray experiments uses a microarray having binding sites corresponding to at least eighty-five percent of the genes in the genome of said first biological system, and wherein said first biological system is a cell or a multicellular organism.

72. (New) The computer system of Claim 15 wherein each said first microarray experiment and each said second microarray experiment in each paired differential microarray experiment in said plurality of paired differential microarray experiments uses a microarray

having binding sites corresponding to at least eighty-five percent of the genes in the genome of said first biological system, and wherein said first biological system is a cell or a multicellular organism.

73. (New) The method of Claim 14 wherein each said first microarray experiment and each said second microarray experiment in each paired differential microarray experiment in said plurality of paired differential microarray experiments uses a microarray having binding sites corresponding to at least ninety percent of the genes in the genome of said first biological system, and wherein said first biological system is a cell or a multicellular organism.

74. (New) The computer system of Claim 15 wherein each said first microarray experiment and each said second microarray experiment in each paired differential microarray experiment in said plurality of paired differential microarray experiments uses a microarray having binding sites corresponding to at least ninety percent of the genes in the genome of said first biological system, and wherein said first biological system is a cell or a multicellular organism.

75. (New) The method of Claim 14 wherein each said first microarray experiment and each said second microarray experiment in each paired differential microarray experiment in said plurality of paired differential microarray experiments uses a microarray having binding sites corresponding to at least ninety-nine percent of the genes in the genome of said first biological system, and wherein said first biological system is a cell or a multicellular organism.

76. (New) The computer system of Claim 15 wherein each said first microarray experiment and each said second microarray experiment in each paired differential microarray experiment in said plurality of paired differential microarray experiments uses a microarray having binding sites corresponding to at least ninety-nine percent of the genes in the genome of said first biological system, and wherein said first biological system is a cell or a multicellular organism.

77. (New) The method of claim 25 wherein said first fluorophore and said second fluorophore are selected from the group consisting of Cy2-deoxynucleotide triphosphate, Cy3-deoxynucleotide triphosphate, Cy3.5-deoxynucleotide triphosphate, Cy5-deoxynucleotide triphosphate, Cy5.5-deoxynucleotide triphosphate, Cy7-deoxynucleotide triphosphate, fluorescein, lissamine, phycoerythrin, and rhodamine.

78. (New) The method of claim 26 wherein said single fluorophore is selected from the group consisting of Cy2-deoxynucleotide triphosphate, Cy3-deoxynucleotide triphosphate, Cy3.5-deoxynucleotide triphosphate, Cy5-deoxynucleotide triphosphate, Cy5.5-deoxynucleotide triphosphate, Cy7-deoxynucleotide triphosphate, fluorescein, lissamine, phycoerythrin, and rhodamine.

79. (New) The computer system of Claim 15 wherein each paired differential microarray experiment in said plurality of paired differential microarray experiments is a two-fluorophore microarray experiment wherein a first fluorophore represents said baseline state of said first biological system and a second fluorophore, distinguishable from said first fluorophore, represents said perturbed state of said first biological system.

80. (New) The computer system of claim 79 wherein said first fluorophore and said second fluorophore are selected from the group consisting of Cy2-deoxynucleotide triphosphate, Cy3-deoxynucleotide triphosphate, Cy3.5-deoxynucleotide triphosphate, Cy5-deoxynucleotide triphosphate, Cy5.5-deoxynucleotide triphosphate, Cy7-deoxynucleotide triphosphate, fluorescein, lissamine, phycoerythrin, and rhodamine.

81. (New) The computer system of Claim 15 wherein a single fluorophore is used in said paired differential microarray experiments.

82. (New) The computer system of claim 81 wherein said single fluorophore is selected from the group consisting of Cy2-deoxynucleotide triphosphate, Cy3-deoxynucleotide triphosphate, Cy3.5-deoxynucleotide triphosphate, Cy5-deoxynucleotide triphosphate, Cy5.5-

deoxynucleotide triphosphate, Cy7-deoxynucleotide triphosphate, fluorescein, lissamine, phycoerythrin, and rhodamine.

83. (New) The method of Claim 14 wherein said intensity independent statistic comprises the expression:

$$\frac{(X-Y)}{\sqrt{\sigma_X^2+\sigma_Y^2+f^2(X^2+Y^2)}}$$

where X represents an intensity of said cellular constituent in said first microarray experiment of said reference pair of microarray experiments, Y represents an intensity of said cellular constituent in said second microarray experiment of said reference pair of microarray experiments, σ_X^2 is a variance term for X that represents an additive error level in X, σ_Y^2 is a variance term for Y that represents an additive error level in Y, and f is a fractional multiplicative error level, and

said amount of change in expression level of said cellular constituent between the second microarray experiment and the first microarray experiment is determined using said error distribution statistic by a method comprising:

generating intensity independent contour lines using the denominator of said intensity independent statistic; and

determining the contour level of said change in expression level of said cellular constituent between the second microarray experiment and the first microarray experiment.

84. (New) The method of claim 83, wherein said intensity independent contour lines are gridded at a value selected from the group consisting of ±0.25 standard deviations, ±0.5 standard deviations, ±1 standard deviations, and ±2 standard deviations.

85. (New) The computer system of Claim15 wherein said intensity independent statistic comprises the expression:

$$\frac{(X-Y)}{\sqrt{\sigma_X^2+\sigma_Y^2+f^2(X^2+Y^2)}}$$

where X represents an intensity of said cellular constituent in said first microarray experiment of said reference pair of microarray experiments, Y represents an intensity of said cellular constituent in said second microarray experiment of said reference pair of microarray experiments, σ_X^2 is a variance term for X that represents an additive error level in X, σ_Y^2 is a variance term for Y that represents an additive error level in Y, and f is a fractional multiplicative error level, and

said amount of change in expression level of said cellular constituent between the second microarray experiment and the first microarray experiment is determined using said error distribution statistic by a method comprising:

generating intensity independent contour lines using the denominator of said intensity independent statistic; and

determining the contour level of said change in expression level of said cellular constituent between the second microarray experiment and the first microarray experiment.

86. (New) The method of claim 85, wherein said intensity independent contour lines are gridded at a value selected from the group consisting of ±0.25 standard deviations, ±0.5 standard deviations, ±1 standard deviations, and ±2 standard deviations.

87. (New) The method of Claim 14 wherein a radioactive label is used in each said paired differential microarray experiment in said plurality of paired differential microarray experiments.

88. (New) The method of Claim 14 wherein a first radioactive label represents a baseline state of said first biological system in each paired differential microarray experiment in said plurality of paired differential microarray experiments, and a second radioactive label represents a perturbed state of said first biological system in each paired differential microarray experiment in said plurality of paired differential microarray experiments, and wherein said first and second radioactive label have distinct emission spectra.

89. (New) The computer system of Claim 15 wherein a radioactive label is used in each said paired differential microarray experiment in said plurality of differential microarray experiments.

90. (New) The computer system of Claim 15 wherein a first radioactive label represents a baseline state of said first biological system in each paired differential microarray experiment in said plurality of paired differential microarray experiments, and a second radioactive label represents a perturbed state of said first biological system in each paired differential microarray experiment in said plurality of paired differential microarray experiments, and wherein said first and second radioactive label have distinct emission spectra.

91. (New) The computer system of Claim 21 wherein said rank based method comprises computing

$$P(H_0^+)=\prod_i P_i$$

where P_i is the percentile rank of the expression of said cellular constituent in the i^{th} pair of paired differential microarray experiments in said plurality of paired differential microarray experiments, and $P(H_0^+)$ is the chance that said cellular constituent is not up-regulated in said plurality of paired differential microarray experiments.

92. (Amended) The computer system of Claim 15 wherein said rank based method determines a probability that said cellular constituent is down-regulated in response to a perturbation.

93. (New) The computer system of Claim 92 wherein said rank based method comprises computing

$$P(H_0^-) = \prod_i (1 - P_i)$$

where P_i is the percentile rank of the expression of said cellular constituent in the i^{th} pair of paired differential microarray experiments in said plurality of paired differential microarray

experiments, and $P(H_0^-)$ is the chance that said cellular constituent is not up-regulated in said plurality of paired differential microarray experiments.

94. (New) The method of claim 14 further comprising, prior to step (a), performing each said paired differential microarray experiment in said plurality of paired differential microarray experiments to obtain measurements of the expression level of each cellular constituent in said set of cellular constituents.

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